

## ON THE DECREASE OF PERMEABILITY DUE TO CERTAIN BIVALENT KATIONS

W. J. V. OSTERHOUT

(WITH ELEVEN FIGURES)

It has been shown<sup>1</sup> that while NaCl and many other salts of monovalent metals increase the permeability of protoplasm, CaCl<sub>2</sub> has the opposite effect. This effect of CaCl<sub>2</sub> is not permanent; if the exposure be sufficiently prolonged, it will be found that it gradually passes away and is followed by an increase of permeability. The question arises, do other bivalent kations behave like calcium?

The method employed in this investigation was to make determinations of the electrical resistance of living tissues of *Laminaria saccharina* in the manner described in a previous paper.<sup>1</sup> Such determinations afford an accurate measure of the permeability of the protoplasm.

The following experiment<sup>2</sup> will illustrate the effects of CaCl<sub>2</sub>. The resistance of a cylinder of tissue in sea water at 18°C. was found to be 1000 ohms. It was transferred to CaCl<sub>2</sub>, 0.278M, which had the same conductivity as the sea water. After 15 minutes the resistance in CaCl<sub>2</sub> at 18° was found to be 1490 ohms. After 30 minutes more the resistance was still 1490 ohms. Another reading taken 105 minutes later showed the resistance to be 950 ohms; 75 minutes later it was 650 ohms. During this time the control in sea water had not altered its resistance. The results are shown in table I and fig. 1.

The resistance at the beginning of the experiment was 1000 ohms; subtracting from this the resistance of the apparatus (250 ohms) gives the net resistance or the actual resistance of the tissue, which is  $1000 - 250 = 750$  ohms. The net conductance was  $1 \div 750$

<sup>1</sup> Science N.S. 35:1112. 1912.

<sup>2</sup> The chemicals used were the best obtainable and in nearly all cases were Kahlbaum's. The solutions unless otherwise stated were neutral to litmus. This is important, for it has been shown in a previous paper acid may cause a rise in resistance.



=0.00133 mho. After 15 minutes in  $\text{CaCl}_2$  the net resistance was  $1490 - 250 = 1240$  ohms, and the net conductance  $1 \div 1240 = 0.00081$  mho. We may regard the permeability as equal to the conductivity, or, in this case, for convenience, as equal to the conductance. The decrease in permeability was therefore  $0.00133 - 0.00081 = 0.00052$  mho or 39.1 per cent.

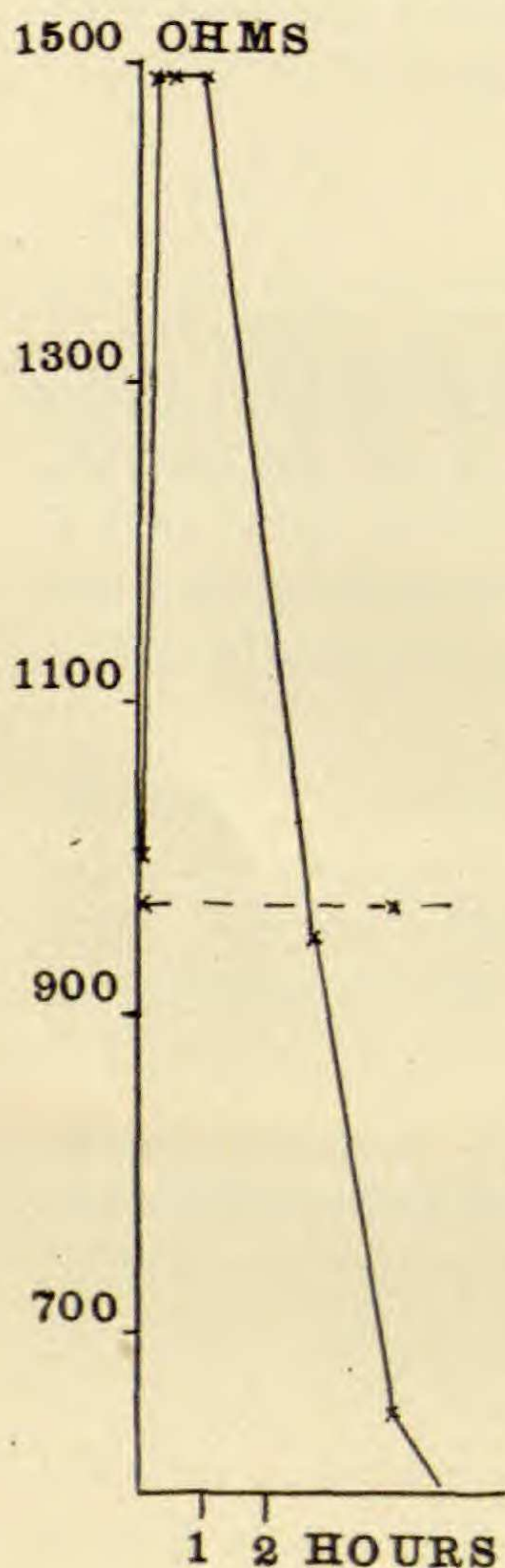


FIG. 1.—Curve of electrical resistance of *Laminaria saccharina* in  $\text{CaCl}_2$  0.278 M (unbroken line) and of a control in sea water (dotted line).

TABLE I

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In $\text{CaCl}_2$ 0.278 M	In sea water
0*	1000	970
$\frac{1}{4}$	1490	970
$\frac{1}{2}$	1490	970
1	1490	970
$2\frac{3}{4}$	950	970
4	650	970

All readings were taken at  $15^\circ \text{C}$ .

The characteristic effects of  $\text{CaCl}_2$  are therefore a very rapid rise followed after an interval by a fairly rapid fall of resistance. It seems probable that these effects result from two processes which go on simultaneously and represent different reactions, one of which has a much greater velocity than the other. In this way the period of stationary resistance (represented by the flattened top of the curve) would be accounted for. The fall in resistance is much slower than that caused by monovalent cations and is, in the opinion of the writer, quite different from it.

Similar results were obtained with  $\text{BaCl}_2$  and  $\text{SrCl}_2$ .

The behavior of material in a solution of  $\text{MgCl}_2$ , about 0.28 M, having the same conductivity as sea water, is shown in table II and fig. 2. The rise in resistance is not nearly as great as in  $\text{CaCl}_2$ ; the fall in resistance begins much sooner and proceeds much more rapidly. The top of the curve is not flattened.



The writer interprets this to mean that the velocity of the second reaction (causing the fall in resistance) is approximately equal to that of the first reaction (causing a rise in resistance).

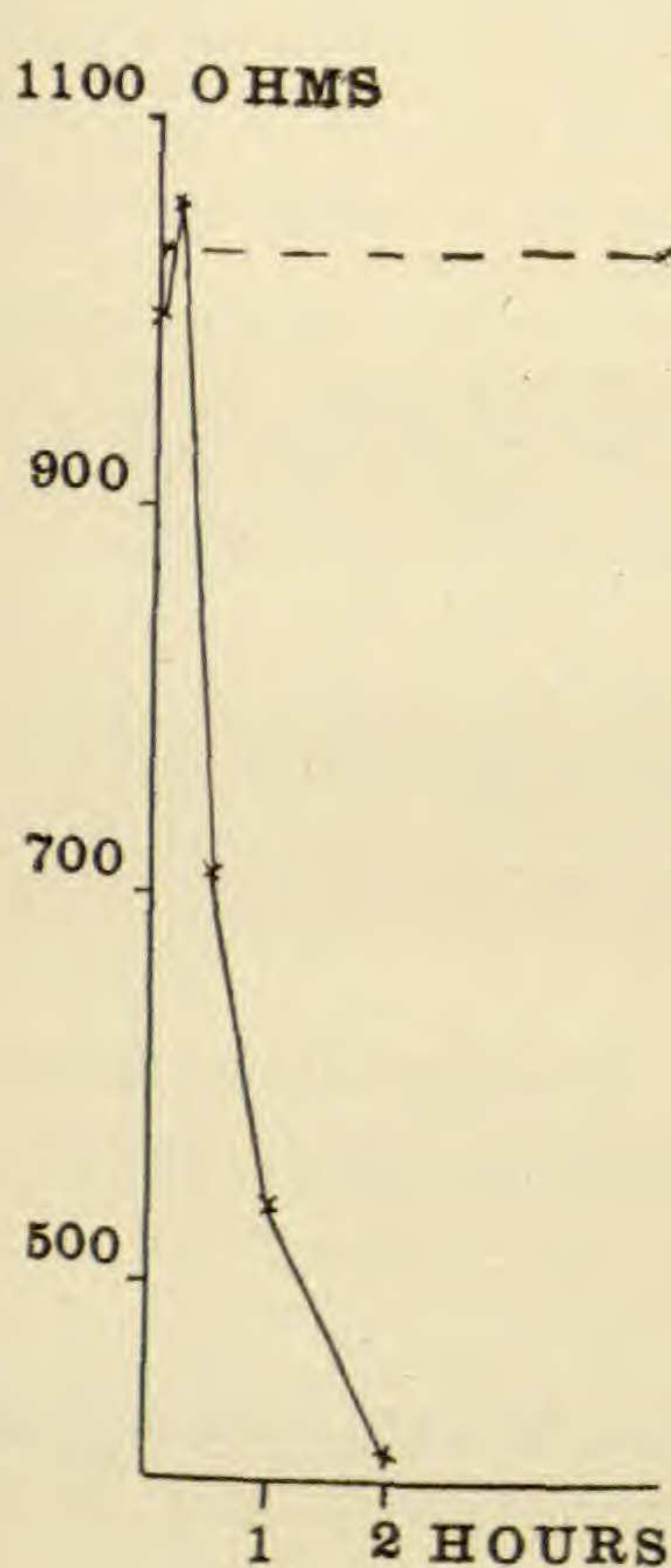


FIG. 2

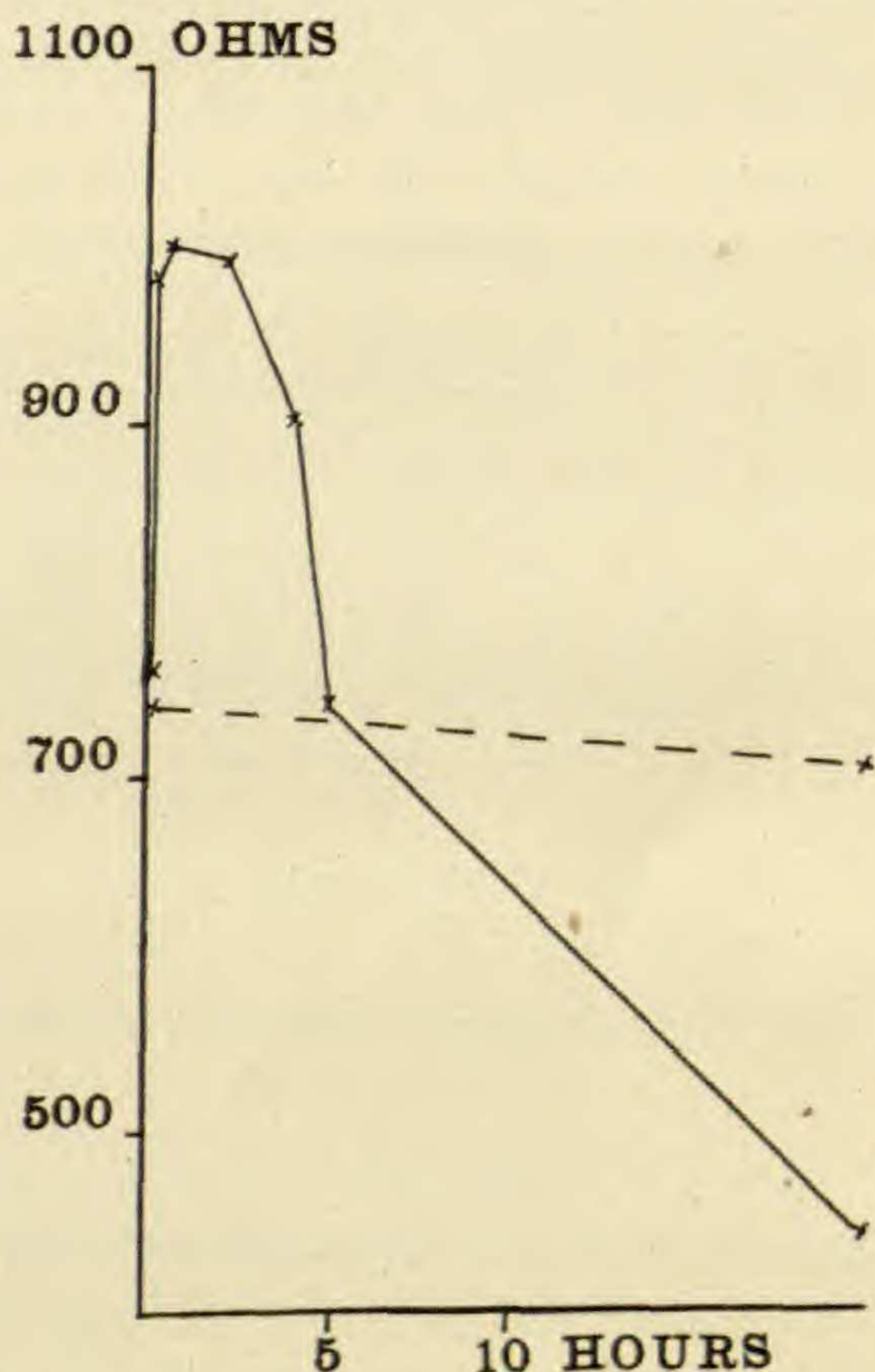


FIG. 3

FIGS. 2, 3.—Fig. 2, curve of electrical resistance of *Laminaria saccharina* in  $MgCl_2$  0.28 M (unbroken line) and of a control in sea water (dotted line); fig. 3, curve of electrical resistance of *Laminaria saccharina* in  $MnCl_2$  0.317 M (unbroken line) and of a control in sea water (dotted line).

TABLE II

ELECTRICAL RESISTANCE OF *Laminaria saccharina*; AVERAGE OF THREE EXPERIMENTS

Time in minutes	In $MgCl_2$ 0.28 M	In sea water
0	1000	1030
10	1055	....
30	710	....
60	545	....
120	410	1030

All readings were taken at 18° C.



In a solution of  $\text{MnCl}_2$  (about 0.317 M), having the same conductivity as sea water, the tissue shows a rapid and very decided rise, followed by a fall which is noticeably slower than that in  $\text{CaCl}_2$ . The results, as shown in table III and fig. 3, are in marked contrast to those obtained with  $\text{MgCl}_2$ .

TABLE III  
ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In $\text{MnCl}_2$ 0.317 M	In sea water
0	760	740
$\frac{1}{4}$	980	...
$\frac{1}{2}$	1000	...
2	990	...
4	900	...
5	740	...
20	440	700

All readings were taken at 18° C.

The rise in resistance in these solutions was so great that it seemed to the writer that a rise might be obtained when the substances in question were added directly to the sea water, either as concentrated solutions or in solid form. Accordingly 10 cc. of  $\text{CaCl}_2$  5 M were added to 1000 cc. of sea water and a lot of tissue was placed in it. The resistance rose<sup>3</sup> from 1290 to 1390 ohms, where it remained stationary for a long time and then began to fall. When the same experiment was tried on dead tissue the resistance fell at once and remained stationary indefinitely. The results are shown in tables IV and IVa and fig. 4. The addition of solid anhydrous  $\text{CaCl}_2$  gave a similar result, although the rise was not as great.

TABLE IV  
ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 1000 cc. + $\text{CaCl}_2$ 5 M 10 cc.	In sea water
0	1290	1320
$\frac{1}{3}$	1280	1320
$\frac{2}{3}$	1300	....
2	1380	1320
$2\frac{1}{3}$	1390	....
13	1390	1320

All readings were taken at 18° C.

<sup>3</sup> The temporary fall in resistance at the start was due to the increased conductivity of the solution contained in the apparatus and in the intercellular substance.



This experiment has a special interest as affording positive proof that the current passes through the protoplasm as well as through the intercellular substance,<sup>4</sup> for it is evident that the rise in resistance in these experiments can not be due to any cause other than a change in permeability of the protoplasm. The concentration of the ions of the sea water remains unchanged, and if they were able to penetrate as freely as they did before the addition of the

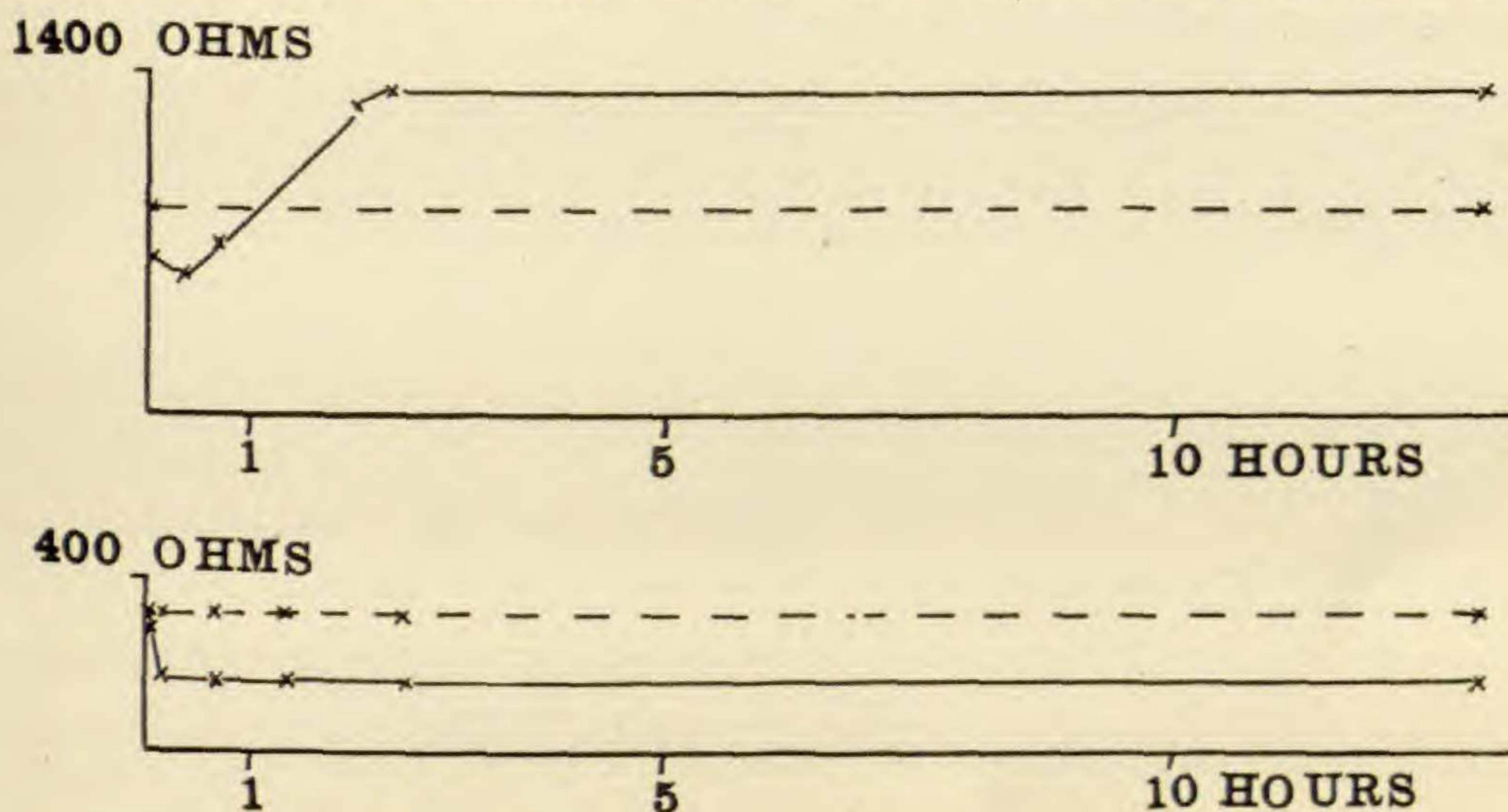


FIG. 4.—Curves of electrical resistance of live tissue (upper figure) and dead tissue (lower figure) of *Laminaria saccharina* in 1000 cc. sea water + 10 cc. CaCl<sub>2</sub> 5 M (unbroken line in both figures) and of controls in sea water (dotted line in both figures).

TABLE IVa

ELECTRICAL RESISTANCE OF DEAD TISSUE OF *Laminaria saccharina*

Time in hours	In sea water 1000 cc. + CaCl <sub>2</sub> 5 M 10 cc.	In sea water
0	370	380
$\frac{1}{6}$	340	380
$\frac{2}{3}$	340	380
$1\frac{1}{3}$	340	380
$2\frac{1}{2}$	340	380
13	340	380

All readings were taken at 18° C.

CaCl<sub>2</sub> the resistance would not increase. It would, in fact, diminish on account of the increased conductivity of the solution held in the intercellular substance, as is clearly shown by experiments on dead tissue. That the change in permeability is in the

<sup>4</sup> The frond may be regarded as a mass of intercellular substance in which the protoplasmic masses are imbedded.



protoplasm and not in the intercellular substance is clearly shown by the fact that as soon as the protoplasm is killed, no rise is produced on adding solid  $\text{CaCl}_2$ . This is true when the means of killing is such as to produce no change in the intercellular substance, e.g., by slight reduction of the water content by partial drying, by allowing the material to stand in the laboratory until dead, or by raising the temperature to  $45^\circ\text{C}$ .

If the rise in resistance were not due to a change in permeability, it could be explained only as the result of a decrease in the size of

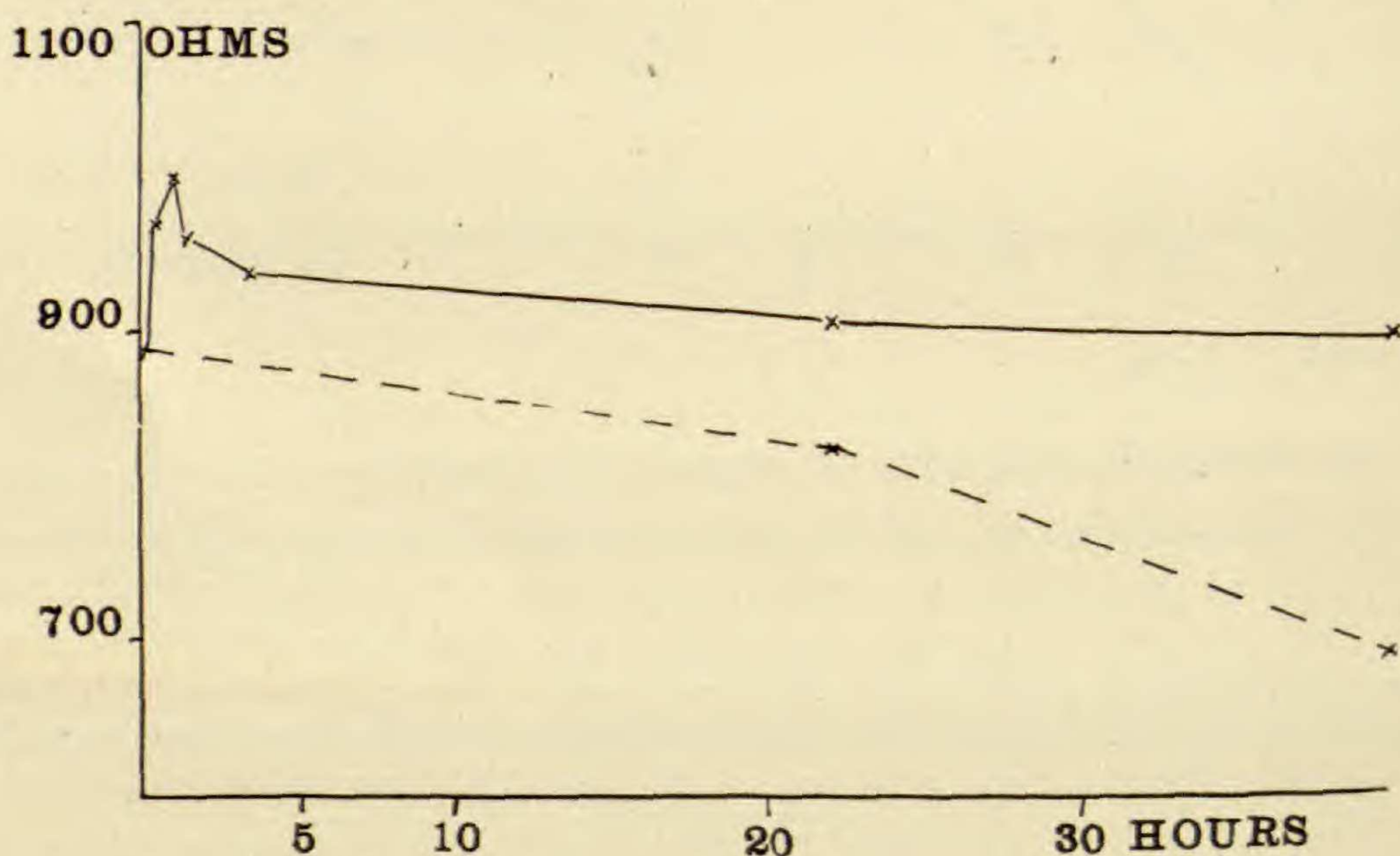


FIG. 5.—Curve of electrical resistance of *Laminaria saccharina* in sea water 1000 cc. +  $(\text{MnSO}_4 + 7\text{H}_2\text{O})$  1.39 gm. = (0.005 M) (unbroken line), and of a control in sea water (dotted line).

the spaces between the cells. Both macroscopic and microscopic measurements show most conclusively that this does not occur. The contrary effect would be produced by the addition of salts in solid form, for they would tend to produce plasmolysis and thereby to increase the space between the cells.

In order to test further the effect of  $\text{MgCl}_2$ , 10 cc. of a 5 M solution were added to 1000 cc. of sea water. A reading taken 5 minutes later showed that the resistance had fallen from 780 to 700 ohms; it continued to fall slowly throughout the experiment. The writer interprets this as showing that  $\text{MgCl}_2$  is not able to



produce sufficient rise to overcome the effect of the increase in the conductivity of the solution which is contained in the intercellular substance.

The addition of  $\text{MnSO}_4$  in solid form produced a decided rise as shown in table V and fig. 5. The amount added was sufficient to make the concentration 0.005 M.

TABLE V  
ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 1000 cc. + $\text{MnSO}_4$ 1.39 gm. (=0.005 M)	In sea water
0	890	890
$\frac{1}{2}$	970	...
1	1000	...
$1\frac{1}{2}$	960	...
$3\frac{1}{2}$	940	...
22	910	830
40	900	690

All readings were taken at 18° C.

It is evident that the addition (to sea water) of a salt in solid form is the severest possible test of its ability to produce a rise in resistance. In subsequent tests of other bivalent kations this method was exclusively employed.

In table VI and fig. 6 are shown the results of two experiments with  $\text{CaCl}_2 + 6 \text{H}_2\text{O}$  which was added to sea water in sufficient amount to make the concentration 0.005 M (1.19 gm. to 1000 cc. sea water). The course of the curves is not quite the same, the lower curve falling more rapidly than the upper. This difference is too great to be the result of experimental error and must be attributed to laboratory conditions and to differences in the material itself, which shows some variation in this respect unless gathered and cut at the same time. It will be noticed that the resistance of the control falls off more rapidly in the lower curve, which must be attributed largely to laboratory conditions. Dead tissue under the same conditions showed no rise in resistance.



TABLE VI  
ELECTRICAL RESISTANCE OF *Laminaria saccharina*:  
TWO EXPERIMENTS

Time in hours	In sea water 1000 cc. (CaCl <sub>2</sub> +6H <sub>2</sub> O) 1.19 gm. (=0.005 M)	In sea water
0	930	890
$\frac{1}{4}$	1010	...
$\frac{1}{2}$	1020	...
1	1010	...
3	980	...
6	960	...
21	940	880
40	900	850
0	940	900
$\frac{1}{4}$	990	...
$\frac{1}{2}$	1020	...
1	1000	...
2	950	...
18	900	...
40	770	780

All readings were taken at 18° C.

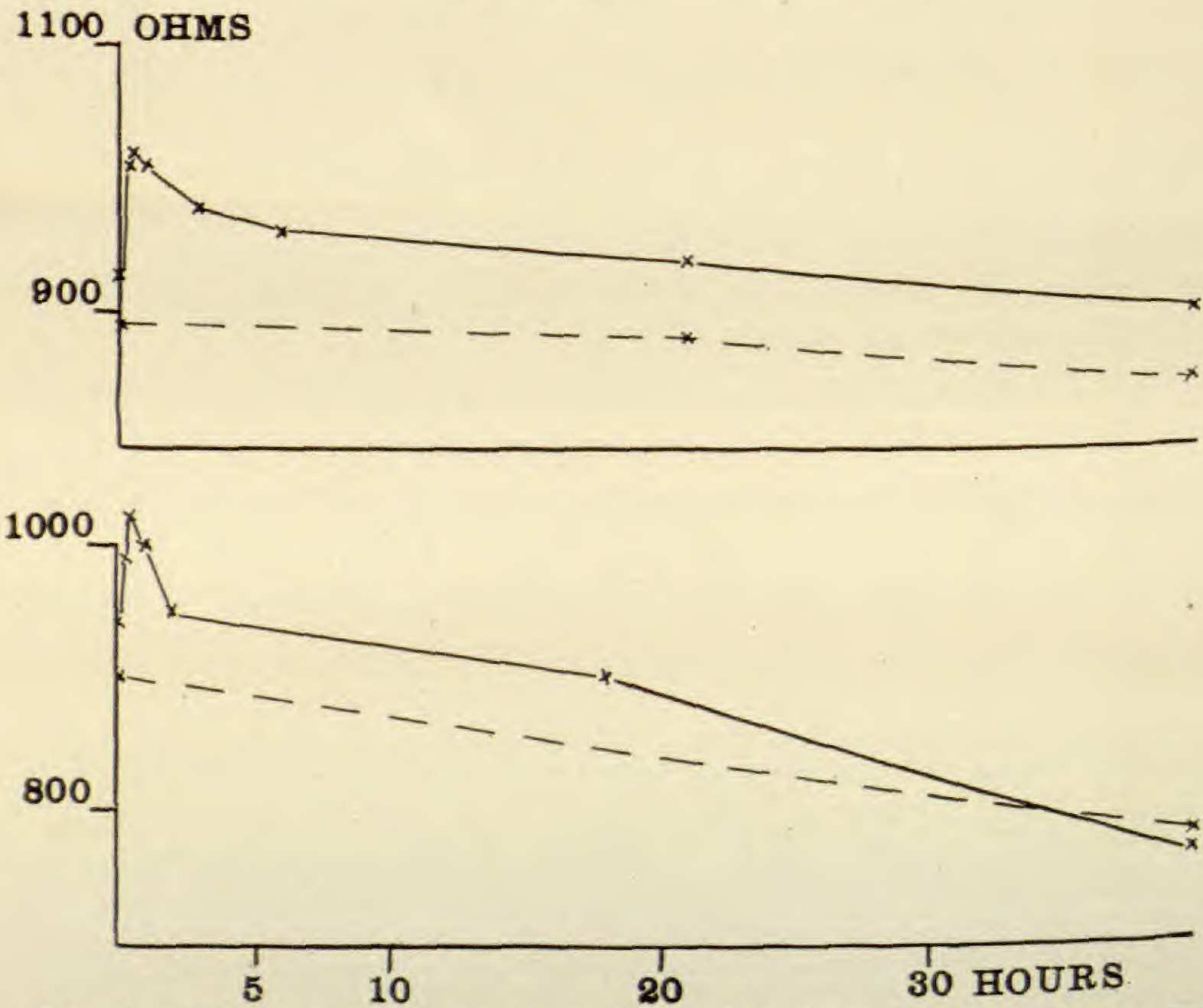


FIG. 6.—Curves of electrical resistance of *Laminaria saccharina* in sea water 1000 cc.+(CoCl<sub>2</sub>+6H<sub>2</sub>O) 1.19 gm. (=0.005 M) (unbroken lines); two experiments; control in sea water (dotted lines).



Table VII and fig. 7 show the behavior of tissue in sea water to which sufficient  $\text{FeSO}_4 + 7\text{H}_2\text{O}$  had been added to make the concentration  $0.005\text{ M}$  (1.39 gm. to 1000 cc. sea water). Some precipitate formed after standing.

TABLE VII  
ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 1000 cc. ( $\text{FeSO}_4 + 7\text{H}_2\text{O}$ ) 1.39 gm. (=0.005 M)	In sea water
0	910	890
$\frac{1}{2}$	1100	...
$\frac{1}{4}$	1040	...
$\frac{1}{2}$	1000	...
$2\frac{1}{4}$	970	...
18	750	780
40	450	730

All readings were taken at  $18^\circ\text{C}$ .

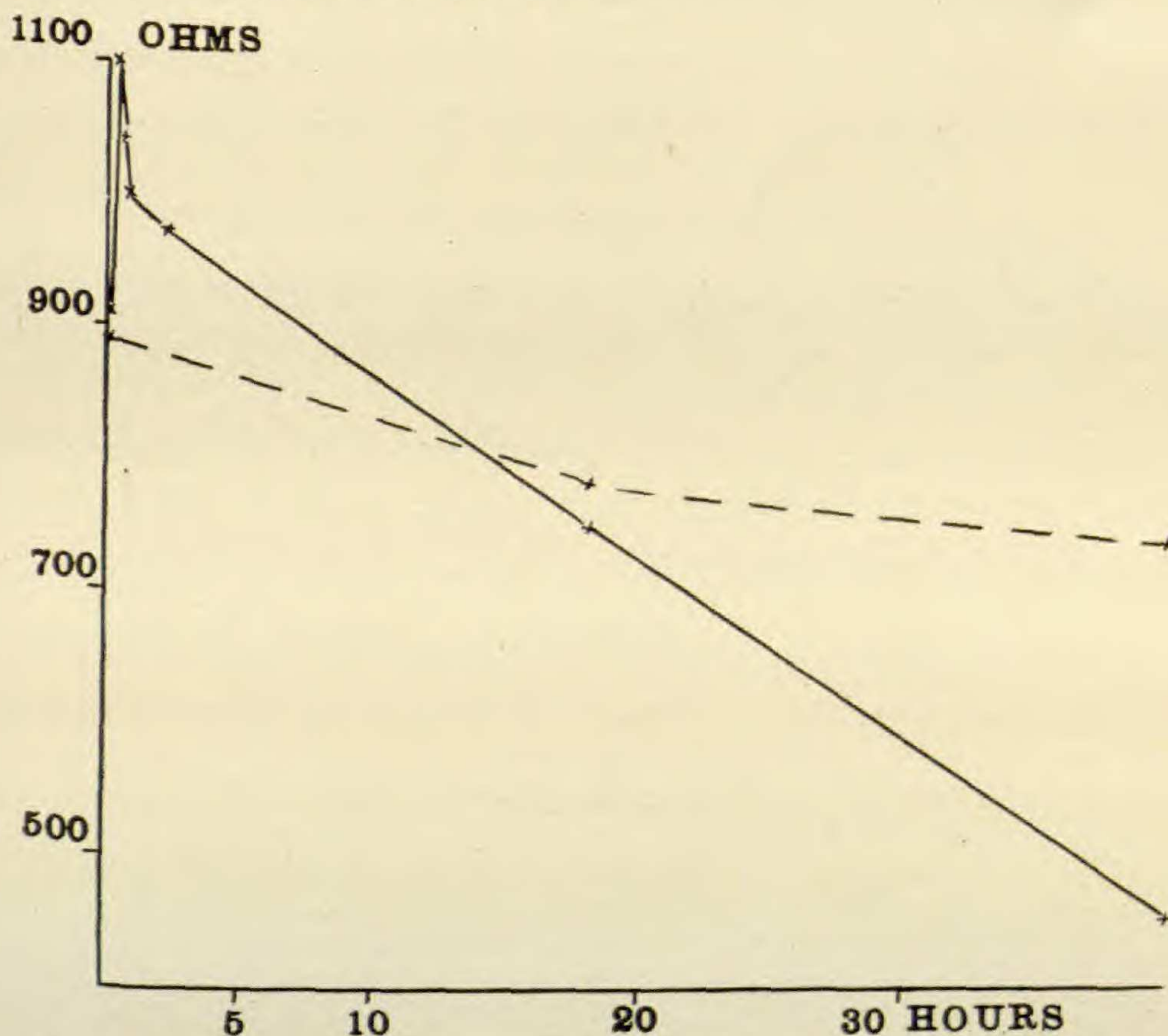


FIG. 7.—Curve of electrical resistance of *Laminaria saccharina* in sea water 1000 cc. + ( $\text{FeSO}_4 + 7\text{H}_2\text{O}$ ) 1.39 gm (=0.005 M) (unbroken line) and of a control in sea water (dotted line).



It will be noticed that while there is a very rapid and decided rise, the fall is much more rapid than in any of the previous experiments in which solid salts had been added to sea water. Neither of these effects can be attributed to acid, as the solution was neutral to litmus. After 18 hours the resistance was below that of the control and it continued to fall rapidly to the death point. Dead tissue showed no rise in resistance.

Table VIII and fig. 8 show the results of experiments in which sufficient  $\text{NiCl}_2 + 6\text{H}_2\text{O}$  was added to the sea water to make the

TABLE VIII  
ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 1000 cc. ( $\text{NiCl}_2 + 6\text{H}_2\text{O}$ ) 1.10 gm (=0.005 M)	In sea water
0	940	890
$\frac{1}{4}$	1000	...
$\frac{3}{4}$	1060	...
1	1020	...
2	1000	...
5	950	...
21	920	840
44	760	600

All readings were taken at 18° C.

concentration 0.005 M (1.19 gm. to 1000 cc. sea water). The results are similar to those obtained with  $\text{CoCl}_2 + 6\text{H}_2\text{O}$ . Dead tissue showed no rise in resistance.

Contrary to expectation the experiments with  $\text{ZnSO}_4 + 7\text{H}_2\text{O}$  showed that it was less toxic than  $\text{FeSO}_4 + 7\text{H}_2\text{O}$ . The results are shown in table IX and fig. 9.

TABLE IX  
ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 1000 cc. ( $\text{ZnSO}_4 + 7\text{H}_2\text{O}$ ) 1.44 gm. (=0.005 M)	In sea water
0	870	860
$\frac{1}{4}$	940	...
$\frac{1}{2}$	950	...
1	990	...
$1\frac{1}{2}$	970	...
3	920	...
19	830	720
40	700	640

All readings were taken at 18° C.



The concentration used was  $0.005\text{ M}$  (1.44 gm. to 1000 cc. sea water).

The curve obtained by using  $\text{CdCl}_2 + 2\text{H}_2\text{O}$  is of a somewhat different type from those previously met with. The rise is not as

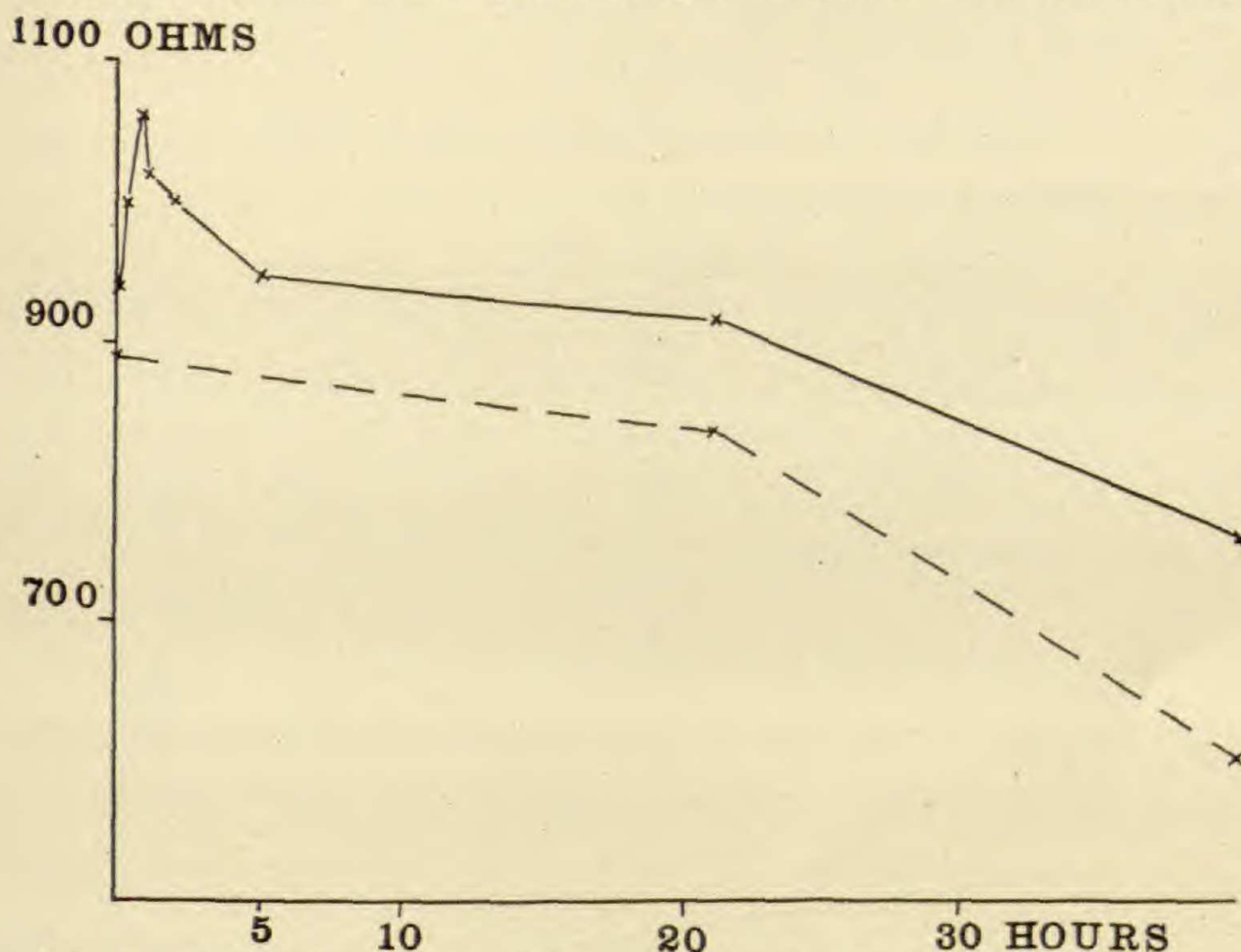


FIG. 8.—Curve of electrical resistance of *Laminaria saccharina* in sea water 1000 cc. +  $(\text{NiCl}_2 + 6\text{H}_2\text{O})$  1.19 gm. ( $=0.005\text{ M}$ ) (unbroken line) and of a control in sea water (dotted line).

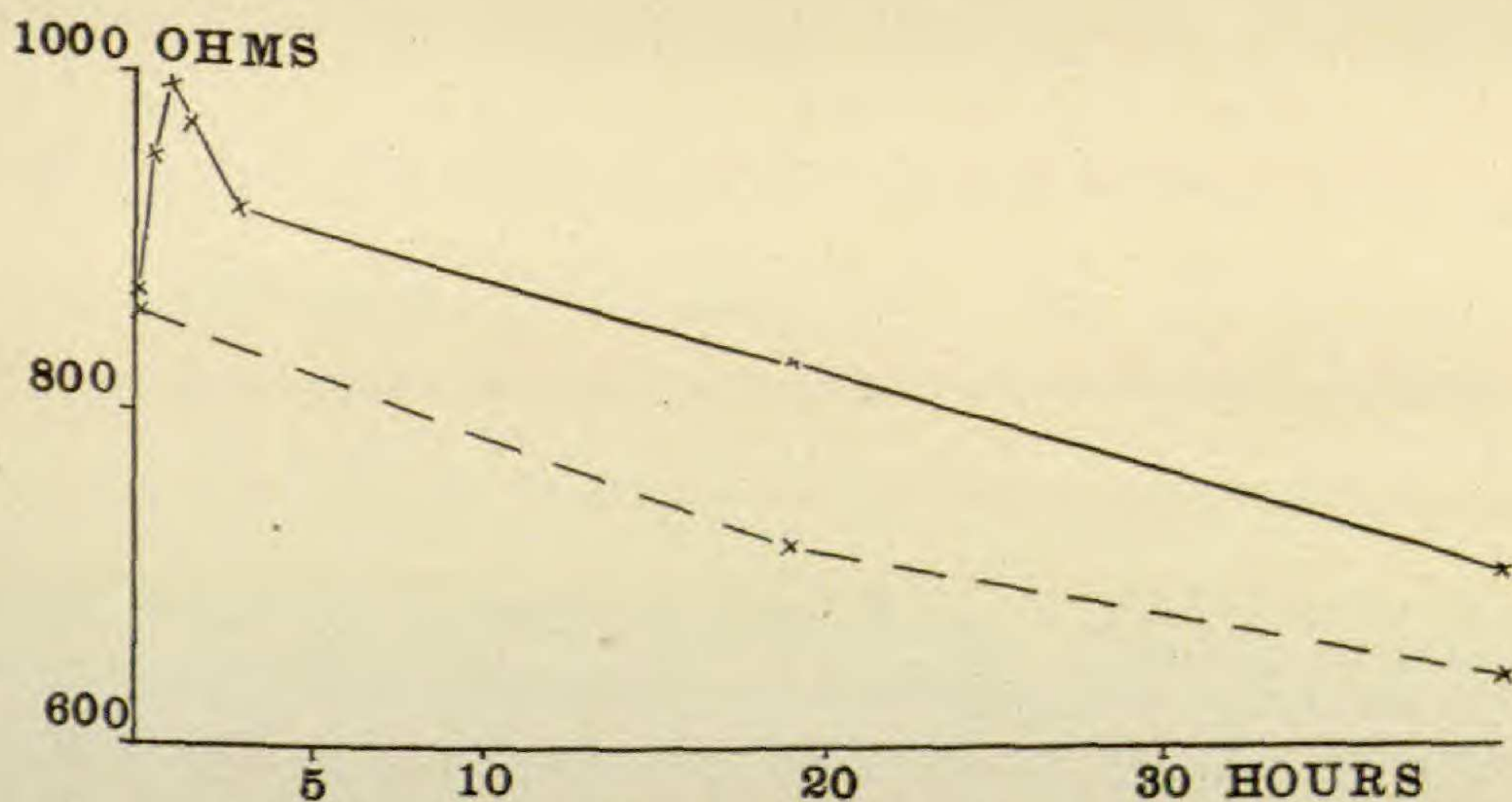


FIG. 9.—Curve of electrical resistance of *Laminaria saccharina* in sea water 1000 cc. +  $(\text{ZnSO}_4 + 7\text{H}_2\text{O})$  1.44 gm. ( $=0.005\text{ M}$ ) (unbroken line) and of a control in sea water (dotted line).



rapid nor as great and the fall is slow, especially at first. The concentration was 0.005 M (1.1 gm. to 1000 cc. sea water). The results are shown in table X and fig. 10. Dead tissue showed no rise in resistance.

TABLE X  
ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 1000 cc. (CdCl <sub>2</sub> +2H <sub>2</sub> O) 1.13 gm. (=0.005 M)	In sea water
0	850	860
$\frac{1}{4}$	900	...
1	920	...
2	930	...
$9\frac{1}{2}$	910	840
$22\frac{1}{2}$	870	820
45	600	680

All readings were taken at 18° C.

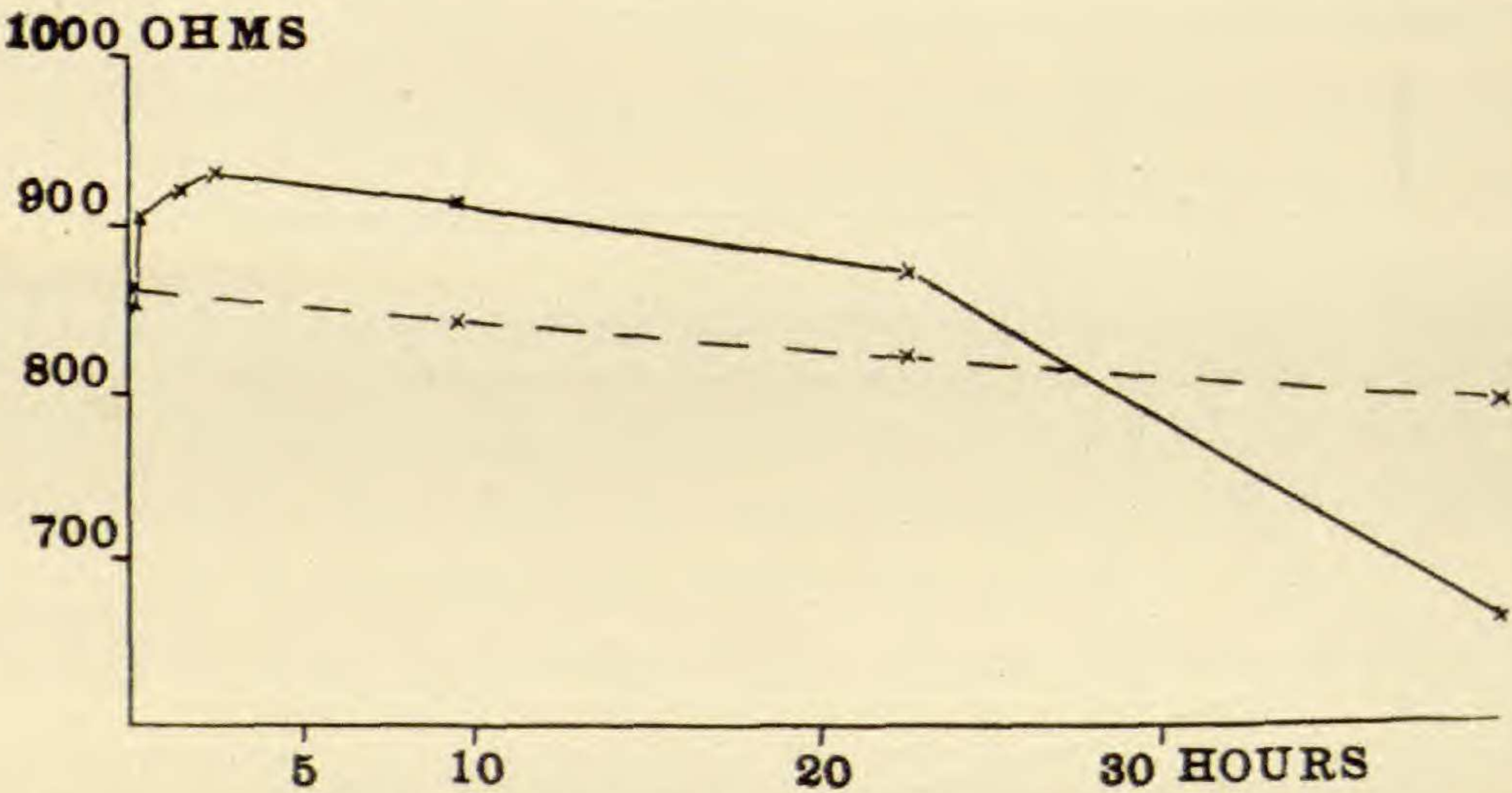


FIG. 10.—Curve of electrical resistance of *Laminaria saccharina* in sea water 1000 cc.+(CdCl<sub>2</sub>+2H<sub>2</sub>O) 1.1 gm. (=0.005 M) (unbroken line) and of a control in sea water (dotted line).

A very different type of curve is obtained by using SnCl<sub>2</sub>+2H<sub>2</sub>O. The rise is rapid and decided and the fall is much more rapid than with any of the other substances used. The solution was acid to litmus, but the effect cannot be considered as due to the acidity alone. Dead tissue showed no rise in resistance.



Some precipitate formed on standing. The concentration used was 0.005 M (1.13 gm. to 1000 cc. sea water).

TABLE XI  
ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 1000 cc. ( $\text{SnCl}_2 + 2\text{H}_2\text{O}$ ) 1.13 gm. (=0.005 M)	In sea water
0	780	750
$\frac{1}{4}$	860	...
$\frac{1}{2}$	870	...
$\frac{3}{4}$	880	...
1	830	...
$1\frac{1}{2}$	750	...
17	400	690
40	320	530

All readings were taken at 18° C.

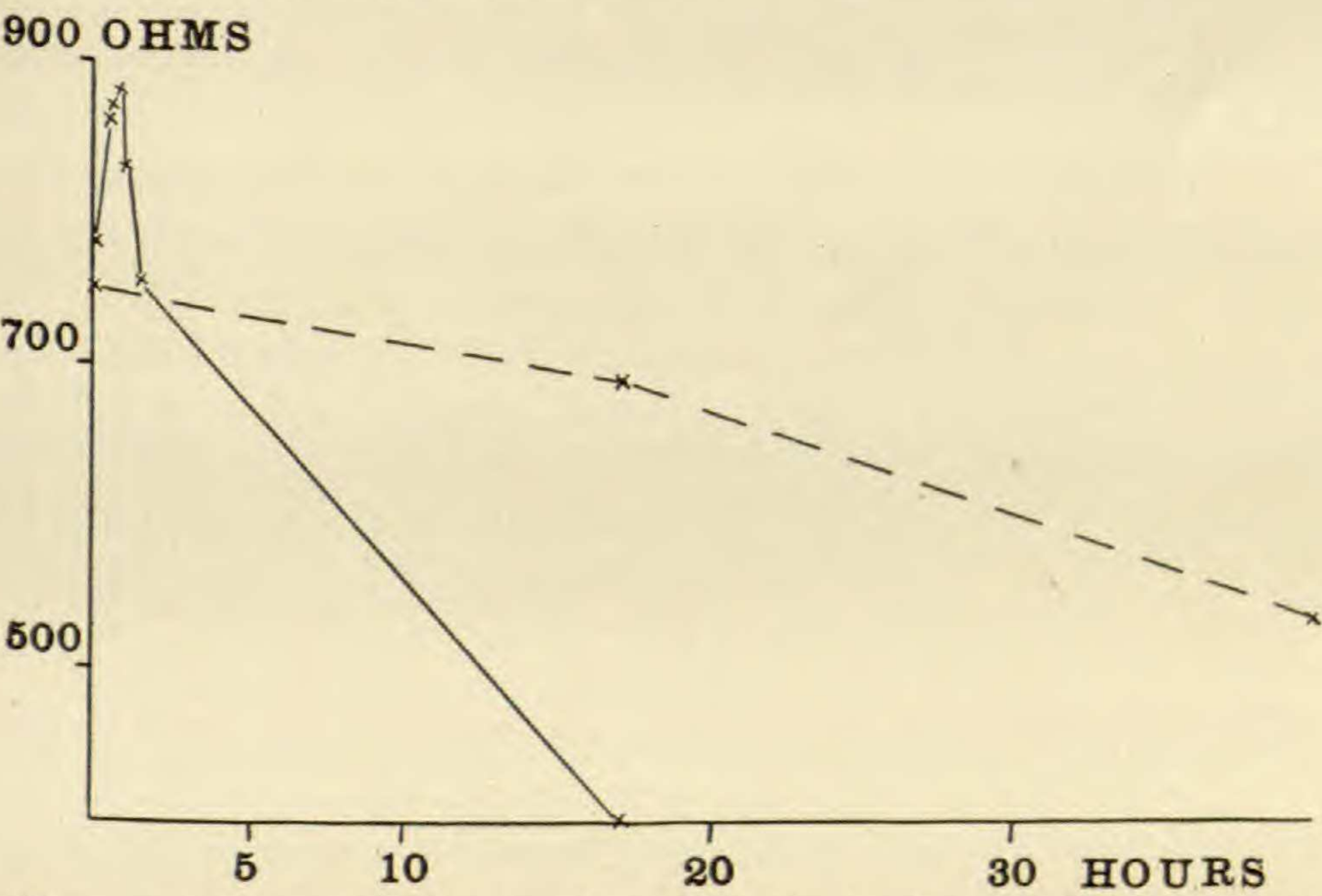


FIG. 11.—Curve of electrical resistance of *Laminaria saccharina* in sea water 1000 cc. + ( $\text{SnCl}_2 + 2\text{H}_2\text{O}$ ) 1.13 gm. (=0.005 M) (unbroken line) and of a control in sea water (dotted line).

It is evident that there is a remarkable difference between the chlorides of monovalent and the chlorides of bivalent kations in their effects on permeability. So far as the writer's experiments have gone, none of the chlorides of monovalent kations are able to decrease permeability (with the significant exception of HCl),



while all of the chlorides of bivalent kations are able to do so to a marked degree. Various tempting hypotheses are suggested by these striking facts. None of them can be worked out at present in a manner which is free from objection, and the writer deems it advisable to defer discussion of them until further investigations have been made.

### Summary

There is a remarkable difference between monovalent and bivalent kations in their effects on permeability.

While none of the monovalent kations (except H) are able to decrease permeability, all the bivalent kations so far investigated (Mg, Ca, Ba, Sr, Mn, Co, Fe, Ni, Zn, Cd, Sn) are able to do so to a marked degree.

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